PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT EXAMINING OPERATION

Applicant(s): Glen H. ERIKSON et al.

Serial No: 09/664,827 Group Art Unit: 1637

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Att. Docket No.: E1047/20044 Confirmation No.: 4947

For: QUADRUPLEX DNA AND DUPLEX PROBE SYSTEMS

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Washington, DC 20231

Sir:

I, J. Hans van de Sande, Ph.D., a citizen of Canada, hereby declare and state:

1. The curriculum vitae attached as Exhibit A accurately reflects my professional credentials. As noted in Exhibit A, I am presently Vice-Dean of the Faculty of Medicine as well as a Professor in the Department of Biochemistry & Molecular Biology (formerly known as the Department of Medical Biochemistry) at the University of Calgary in Calgary, Alberta, Canada.

2. I am a paid consultant of Ingeneus Corp. through my association with Genetic Diagnostics, Inc., a licensee of technology owned by Ingeneus Corp. I expect to be compensated for my time expended preparing this document.

3. Prior to executing this Declaration, I reviewed the above-identified application, the May 30, 2002 Final Rejection, the January 18, 2002 DECLARATION UNDER 37 C.F.R. § 1.132 of Jasmine I. Daksis, and the January 21, 2002 DECLARATION UNDER 37 C.F.R. § 1.132 of Richard A. Collins.

4. The purpose of this Declaration is to address the assertions in the Final Rejection that the application: (a) does not enable one skilled in

the art to make and/or use the invention; and (b) the claimed multiplex structure lacks patentable utility.

Enablement

5. Counsel for Ingeneus Corp. has advised me that an invention is patentably enabled if one of ordinary skill in the art could make or use the invention from the disclosures in the patent application coupled with information known in the art without undue experimentation. While I am not an expert in patent law, my experience and educational background, particularly as a professor of molecular biology and biochemistry, enable me to render an informed opinion as to the facts underlying the determination of enablement, including the level of ordinary skill in the art, information known in the art at the time of the invention, and what constitutes undue experimentation to one of ordinary skill in the art.

6. Claim 1 specifies a multiplex structure comprising four strands, wherein the first strand is associated with the second strand by Watson-Crick bonding, and the fourth strand is associated with the second strand and the third strand by Watson-Crick bonding. In addition, at least one nucleobase of the fourth strand is associated by Watson-Crick bonding to at least one nucleobase of the third strand and to at least one nucleobase of the second strand. The meaning of Watson-Crick bonding in the context of the invention is provided in the application at page 5, lines 25-33:

> As used herein, the term "Watson-Crick bonding" is intended to define specific association between opposing pairs of nucleic acid (and/or nucleic acid analogue) strands via matched, opposing bases. While the formation of a Watson-Crick quadruplex may sometimes be referred to as a hybridization event herein, that is merely for convenience and is not intended to limit the scope of the invention with respect to how the formation of a Watson-Crick quadruplex can be best characterized.

One of ordinary skill in the art would have understood from the foregoing that base claim 1 and the claims dependent therefrom are directed to a quadruplex of four nucleobase-containing strands, wherein Adenines align with Thymines (or Uracils) and Cytosines align with Guanines.

7. The working examples of the specification show that Applicants were able to make and use the invention without undertaking detailed biophysical studies. Applicants have shown through binding studies specific association of non-denatured dsDNA targets with non-denatured dsDNA probes. The concentration of KCl used in, e.g., Example 1 of the application was sufficiently high that it was highly unlikely that strand displacement occurred or that the dsDNA probes or dsDNA targets were denatured in any way. It is known that high concentrations of salt (e.g., 100 mM NaCl) inhibit strand invasion by virtue of the salt increasing the stability of dsDNA. See, e.g., Tomac et al., "Ionic Effects on the Stability and Conformation of Peptide Nucleic Acid Complexes," J. Am. Chem. Soc. 118, 5544-5552 (1996) (previously made of record in the application on January 18, 2002). Accordingly, one of ordinary skill in the art would have expected the 100 mM KCl concentration of Example 1 to prevent strand displacement and denaturation.

8. In light of Applicants' evidence that two strands on opposing non-denatured duplexes specifically interact together A:T(U) and C:G, one of ordinary skill in the art would have found it reasonable to infer that adjacent bases in the remaining two strands of the duplexes would be brought into close enough proximity by the initial pairing of opposing strands to specifically interact together A:T(U) and C:G. This inference would not have

been considered by an ordinarily skilled artisan to be unreasonable, particularly in view of the teachings of, e.g., Zhang et al., "Dimeric DNA Quadruplex Containing Major Groove Aligned A•T•A•T and G•C•G•C Tetrads Stabilized by Inter-subunit Watson-Crick A•T and G•C Pairs," 312 J. Mol. Biol. 1073-88 (Oct. 5, 2001), attached as Exhibit B.

9. Zhang et al. disproves the theory in the Final Rejection at page 6, last sentence, that "Watson-crick hydrogen bonding surfaces are inaccessible for any other strands [i.e., strands other than the two hybridized strands of conventional duplexes] since two strands are already interacting with each other at the center of the double helix." Zhang et al., shows through NMR studies the formation of A-T-A-T tetrads similar to previously discovered G-C-G-C tetrads. Zhang et al. at pages 1073-74 states:

> [E]fforts have been made to identify and characterize G•C•G•C tetrads, where a pair of Watson-Crick G•C pairs can potentially align either through their major groove or their minor groove edges. . . . recent studies have demonstrated that G•C•G•C tetrads aligned through their major groove edges can switch between two distinct alignment geometries [shown in Figure 1(a) and 1(b)]. . . . The major groove-aligned G•C•G•C tetrad has now been observed in a range of DNA quadruplexes and appears to be a robust tetrad motif adopted by a wide range of DNA sequences.

Figure 1 of Zhang et al. shows how major groove-aligned G•C•G•C and A•T•A•T tetrads in their direct alignment geometry have each G hydrogen bonded to each C, and each A hydrogen bonded to each T. Thus, contrary to the Final Rejection, Zhang et al. and the art cited therein shows that quadruplex G-C-G-C and A-T-A-T binding is reasonably credible.

10. Thus, one of ordinary skill in the art (who has a high level of skill and a high tolerance for complex experimentation) would have been able to make and use with no more than routine experimentation the claimed multiplexes for specific and useful purposes such as assays without ever

knowing for certain the location, length and/or number of hydrogen bonds between adjacent bases in the multiplex.

Utility

11. As shown by Zhang et al., the art recognizes the existence of quadruplex G-C-G-C and A-T-A-T binding under certain conditions, contrary to the assertion in the Final Rejection. Applicants have shown through binding studies specific association of non-denatured dsDNA targets with non-denatured dsDNA probes, wherein the targets and probes align Adenine to Thymine (or Uracil) and Cytosine to Guanine. Accordingly, one of ordinary skill in the art would have found the claimed invention to be reasonably credible in view of the original disclosure of the invention and conventional wisdom in the art.

12. Similarly, the binding studies described in the previously filed Daksis and Collins Declarations further show the reasonable credibility of the claimed quadruplexes through evidence establishing the existence of related Watson-Crick triplexes. Further evidence exists in the form of the inventors' previously issued U.S. Patents Nos. 6,420,115, 6,403,313 and 6,265,170, which include many examples of Watson-Crick triplex binding, wherein Adenines align with Thymines (or Uracils) and Cytosines align with Guanines. Such evidence of triple-stranded Watson-Crick binding undercuts the theory that only duplex Watson-Crick binding exists, and adds to the evidence that Watson-Crick quadruplexes are a reality.

13. In addition, I have personally observed triplex hybridization experiments similar to those described in the working examples of the application, wherein single-stranded probes were able to discriminate between perfectly matched duplex targets and mismatched duplex targets under

non-denaturing conditions. The experiments further convinced me of the high degree of recognition between the single-stranded probe and the duplex target with complete base pairing between binding partners.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date: Sept 24, 2002

J. Hans van de Sande, Ph.D.